# PART 1: SCIENTIFIC ABSTRACT

Angiogenesis, the formation of new blood vessels from existing blood vessels, is a complex physiologic process involving numerous mediators including the angiogenic growth factors Vascular Endothelial Growth Factor (VEGF), basic Fibroblast Growth Factor (bFGF), acidic FGF (aFGF), angiopoietins and others[1]. The term "therapeutic angiogenesis" has been used to describe a strategy employing gene transfer or protein formulations of angiogenic growth factors, which stimulate or augment new blood vessel development, to target vascular insufficiency. Gene transfer, when administered into ischemic tissue, permits targeted delivery of the therapeutic transgene and maintenance of a concentration of the angiogenic protein in that region for days to weeks following a single administration.

HIF-1α (Hypoxia-inducible factor-1 alpha) is an inducible transcriptional regulatory factor that, in combination with its constitutively expressed dimerization partner, HIF-1β plays a principal role in the cellular response to changes in oxygen tension. Through control of a number of genes encoding inducible nitric oxide synthase, VEGF and glycolytic enzymes among others, the HIF-I α complex potentiates and coordinates adaptation to hypoxia at the systemic, tissue, and cellular levels[2,3]. Transcriptional regulation by HIF-1 is mediated by binding of this heterodimeric transcription factor to hypoxia responsive elements (HREs) in the promoter/enhancer regions of target genes[4]. Stabilization of the HIF-1 α subunit accounts for hypoxic induction of HIF-1α DNA binding activity[5,6,7]. This observation, as well as studies in HIF-1α knockout mice, has established that HIF-1α is essential for regulating gene expression in response to changes in oxygen tension both during development and in response to hypoxia in postnatal life[8,9].

It had been reported that a deletion mutant of HIF-1α truncated at amino acid 390 exhibits much reduced transactivational activation but retains a high level of DNA binding that is equivalent in hypoxic and non-hypoxic cells[10]. This result suggested that both the transactivation domain and the region in HIF-1α responsible for conferring destabilization with normoxia are located between amino acids 390 and 826. It has since been shown that this region contains two conserved prolines that are hydroxylated under normoxic conditions, thus targeting the HIF-1α protein for degradation. By deleting this region of HIF-1α and replacing it with the transactivation domain of herpes simplex virus VP16, Genzyme has created a transcription factor capable of sustained activation of genes involved in the cellular response to hypoxia.

Therefore, administration of a modified HIF-1α transcription factor via gene transfer might induce expression of a panel of potentially beneficial genes and ultimately lead to the neovascularization of ischemic tissues. Among the target genes for this modified transcription factor is VEGF[11], an endothelial cell-specific mitogen and potent stimulator of angiogenesis[12]. VEGF gene expression is up-regulated in response to myocardial ischemia[13,14] and therapeutic angiogenesis has been induced in animal models of peripheral and myocardial ischemia through administration of VEGF-A as a recombinant protein or as naked plasmid DNA[15,16,17].

Genzyme has modified the transactivation domain of HIF-1 α to enhance its stability, and developed the Ad2/HIF-1α/VP16 recombinant adenoviral vector in an attempt to maximize the expression of this modified HIF-1α transcription factor within targeted ischemic tissues. *In vitro* analyses documented the activity of plasmid and adenoviral HIF-1α/VP16 construct, and provided the rationale for *in vivo* bioactivity studies. The bioactivity of the HIF-1α/VP16 gene *in vivo* was first demonstrated by injection of plasmid DNA (pDNA) encoding the gene into skeletal muscle in the rabbit ischemic hindlimb model. Plasmid encoding HIF-1α/VP16 (pHIF-1α/VP16) stimulated a level of angiogenesis that was significantly greater than that stimulated by the positive control, a plasmid encoding $VEGF_{165}$ [18].

The initial study in a series of Genzyme experiments to support the peripheral arterial disease (PAD) indication (previously referred to by Genzyme as PVD or peripheral vascular disease) documented the bioactivity of Ad2/HIF-1α/VP16 and compared the bioactivity of Ad2/HIF-1α/VP16 to that of pHIF-1α/VP16. This study was designed to determine the choice of vector (adenovirus or plasmid) to be used in all further preclinical animal studies for PAD and the recently completed Phase 1 clinical trials (NIH protocols 9907-327/-328/-329). Results from this study in the rabbit ischemic hindlimb model demonstrated bioactivity of Ad2/HIF-1α/VP16 that was significantly improved compared to pHIF-1α/VP16. Therapeutic angiogenesis was observed using doses of $\leq 1 \times 10^{10}$ particles of Ad2/HIF-1α/VP16, indicating that a dose lower than $1 \times 10^{10}$ particles of Ad2/HIF-1α/VP16 may stimulate therapeutic angiogenesis in a clinical setting. Preclinical studies assessed safety and toxicity of a single 50 µL IM injection of $1 \times 10^{8}$, $1 \times 10^{9}$, and $1 \times 10^{10}$ viral particles of Ad2/HIF-1α/VP16 into the rat skeletal muscle for as long as 90 days. The main toxicological finding was minimal to mild, apparently dose dependent, microscopic inflammation at the administration site. Systemic toxicity was not observed. More information on the pre-clinical activities that support the

PAD indication is provided in Appendix M-II-B-2. These studies are in addition to the intramyocardial safety and toxicity studies conducted in support of Genzyme's cardiac ischemia clinical protocol (NIH Protocol 0007-407), which also studied Ad2/HIF-1α/VP16.

The initial Ad2/HIF-1α/VP16 clinical study for the PAD indication was a Phase 1, randomized, double-blind, placebo-controlled, dose escalation study conducted in patients with critical limb ischemia (CLI), a severe form of PAD characterized by rest pain and/or non-healing tissue necrosis non responsive to standard measures of care (NIH protocols 9907-327/-328/-329). This study, which was conducted at five clinical sites in the United States between October 1999 and June 2003, involved a total of 34 patients who received Ad2/HIF-1α/VP16 gene transfer. Overall, the results from the PAD Phase 1 program have shown Ad2/HIF-1α/VP16 to be well-tolerated at doses ranging from $1 \times 10^8$ up to $2 \times 10^{11}$ vp in patients with CLI who are no longer candidates for standard revascularization procedures. There were no SAEs related to Ad2/HIF-1α/VP16. Other than mild to moderate injection site reactions, no adverse drug reactions have been identified in these studies. No safety problems emerged which would prevent Ad2/HIF1α/VP16 from being tested in patients with CLI or the proposed indication in the Phase 2 trial, intermittent claudication. The Phase 1 results also demonstrated favorable preliminary clinical outcome measures in this patient population with observations of rest pain resolution and complete ulcer healing.

The proposed Phase 2 clinical study will be conducted in patients with severe intermittent claudication (IC) because it represents a well-defined patient population with a significant unmet medical need. These patients have advanced but stable disease and their exercise capacity is sufficient to test for a dose response with reasonable group sizes. Importantly, the study endpoints for this indication (peak walking time on a walking treadmill test) are widely accepted for proof of concept studies. The patient population will consist of patients with bilateral PAD who have severe intermittent claudication in at least one leg as defined by a walking limitation between 1 and 10 minutes on a walking treadmill test.

Genzyme proposes to conduct a prospective, randomized, double-blind, placebo-controlled, parallel group, multicenter, Phase 2 dose-selection study designed to investigate the safety and efficacy of 3 doses of Ad2/HIF-1α/VP16 for severe IC.

Patients will be randomized to receive either 1 of 3 doses of Ad2/HIF-1α/VP16 gene transfer or Placebo in a 1:1:1:1 ratio. Patient randomization will be stratified based on diabetes mellitus status at Baseline. The 3 total doses of Ad2/HIF-1α/VP16 being evaluated are $2 \times 10^9$, $2 \times 10^{10}$ and $2 \times 10^{11}$ viral particles (vp). Seventy-five patients will be enrolled into each of 4 study drug groups (including the placebo group) for a total of 300 patients. The study drug (gene transfer or placebo) for each patient will consist of a single dose administered intramuscularly (IM) to both lower limbs with 20 injections (100 μL each) in each lower limb for a total of 40 injections.

The duration of each patient's participation in the study will be 2 years. During the administration and initial follow-up, patients will have scheduled visits at Day 1 and Weeks 1, 4, 12, 26, and 52. The primary efficacy endpoint for this study will be the peak walking time (PWT), defined as the maximum time a patient can walk before stopping due to claudication pain, using a standardized walking treadmill test. The analysis of the primary efficacy endpoint will be conducted at Week 26; however, individual patient randomization assignment will remain blinded for the duration of the administration and initial follow-up phase (through Week 52) for the evaluation of safety and secondary endpoints.

During the Extended Safety Follow-up of this protocol, patients will be scheduled for follow-up visits at Weeks 78 (18 months) and 104 (2 years). These visits will focus on assessment of major cardiac and vascular adverse events (AEs). Patients also will be asked to participate in a Long Term Follow-up program for up to 15 years in an effort to identify potential late-occurring toxicities (e.g., *de novo* cancers, and hematological, neurological, and autoimmune disorders) that may be associated with the use of an investigational gene transfer product.

The safety data collected for this Phase 2 dose-selection study include AEs, physical examination findings and vital signs, clinical laboratory parameters (hematology, liver function tests, renal function tests, blood chemistry, urinalysis, prostate-specific antigen [PSA], occult blood in stool), specific cancer screening tests, Ad2 antibody and neutralizing antibody titers, eye exams, and serious adverse events (SAEs) that are major cardiac/vascular AEs. Patient samples (i.e., blood, urine, throat swab, and semen-if feasible) also will be collected to assess the potential for presence of viral shedding.

The efficacy measures will include the primary endpoint of PWT, and other endpoints including claudication onset time (COT) using a standardized exercise treadmill test

protocol, as well as two validated quality of life (QoL) instruments (a global instrument, short form 36 [SF-36], and a disease-specific instrument, the Walking Impairment Questionnaire [(WIQ)]), and ankle-brachial index (ABI).

As part of the conduct of this study, Genzyme will implement a safety evaluation plan to provide an ongoing evaluation of the nature, frequency, and severity of AEs that have been identified as potential side effects associated with the use of Ad2/HIF-1α/VP16 and other new observations. An independent Data Monitoring Committee (DMC) will provide an ongoing, expert, independent review of safety data to assure that the risks to study patients are minimized. This ongoing review will include pre-specified interim analyses of safety data during the study. The DMC will conduct the first interim assessment of patient safety when the first 60 patients have received study drug and been followed for at least 4 weeks, and up to 72 patients have been enrolled in total. Throughout the study, the DMC will receive notification on an expedited basis of all deaths and SAEs considered possibly related to study drug administration. The DMC will conduct the first interim safety assessment on an unblinded basis. At this time, further randomization and administration will be suspended for at least 4 weeks during the interim safety review until authorization to proceed is received from the DMC.

Thereafter, DMC reviews of patient safety will occur in a blinded manner (unless unblinding is specifically requested by the DMC) after each additional cohort of 60 patients (i.e., 120, 180, and 240 patients), enrolled and received study drug, have completed 4 weeks of follow-up. However, in the event that enrollment is slower than anticipated, the time between DMC reviews of patient safety will not exceed 6 months. Suspending enrollment for these reviews only will be required if specified by the DMC.